Application No.: 10/616,082
Amendment Date: 22-Oct-08
Reply to Office Action of: 14 May 2008

REMARKS/ARGUMENTS

Claims 1-2, 6, 10-16, 18, 19, 26-30, and 57-58 are pending. Claims 3, 4, 5, 7, 8, 9, 17, 20-25, and 31-56 are cancelled.

Claims 1 and 2 have been amended to recite that the step comprises the step of expressing in the host cell a nucleic acid encoding a

> chimeric mannosidase enzyme comprising
> (a) a *D. melanogaster* mannosidase II catalytic domain fused to a cellular targeting signal peptide selected from the group consisting of Gls1-s, Mns1-s, Mns1-m, S.Sec-s, S.Sec-m, S.Sec-l, P.Sec-s, P.Sec-m, Mnn9-s, Van1-s, Van1-m, Van1-l, Anp1-s, Anp1-m, Anp1-l, Hoc1-s, Hoc1-m, Hoc1-l, Mnn10-m, Mnn11-s, Mnt1-m, J3-m, Ktr1-s, Ktr2-s, Gnt1-s, Gnt1-m, Gnt1-l, Mnn2-s, Mnn2-m, Mnn2-l, Mnn5-m, Mnn1-s, Mnn1-m, Mnn1-l, Mnn6-s, and Mnn6-m or
> (b) a *C. elegans* mannosidase II catalytic domain fused to a cellular targeting signal peptide selected from the group consisting of Gls1-s, Mns1-s, Mns1-m, S.Sec-s, S.Sec-m, S.Sec-l, P.Sec-s, Van1-s, Van1-m, Van1-l, Anp1-s, Hoc1-m, Mnn10-s, Mnn10-m, Mnn10-l, Mnn11-s, Mnn11-m, Mnt1-s, Mnt1-m, Mnt1-l, D2-s, D2-m, D9-m, J3-m, Ktr2-s, Gnt1-s, Gnt1-m, Mnn2-s, Mnn2-m, Mnn2-l, Mnn5-s, Mnn5-m, Mnn1-s, Mnn1-m, and Mnn6-m,
> wherein said chimeric enzyme in (a) or (b) is capable of hydrolyzing *in vivo* more than 40% of the Man α-1,3 and/or Man α-1,6 linkages of a $GlcNAcMan_5GlcNAc_2$ substrate . . . .

Support for this amendment can be found in Table 11 of Example 14 and paragraph [698]. Additional support can be found in paragraphs [0062], [0063], and [0113]. Table 11 shows that particular chimeric mannosidase II enzymes when expressed in a *Pichia pastoris* host cell resulted in the cell being able to produce glycoproteins wherein at least 40% of the N-glycans had a $GlcNAcMan_3GlcNAc_2$ glycoform. The chimeric enzyme accomplished this by hydrolyzing the Man α-1,3 and Man α-1,6 linkages of the $GlcNAcMan_5GlcNAc_2$ N-glycans on the glycoprotein. Claims 1 and 2 claim only those particular chimeric mannosidase II enzymes.

Claim 10 has been amended to recite an embodiment of claim 1 or 2 wherein the chimeric enzyme comprises a Class IIx mannosidase catalytic domain fused to a cellular targeting signal peptide that targets the chimeric enzyme to the secretory pathway of the host cell instead of the chimeric enzyme of Table 11. Support for the amendment can be found in for example paragraphs [0035] and [0057].

Claim 12 has been amended to recite an embodiment of claim 1 or 2 wherein the chimeric enzyme comprises a Class III mannosidase catalytic domain fused to a cellular targeting signal peptide that targets the chimeric enzyme to the secretory pathway of the host cell

instead of the chimeric enzyme of Table 11. Support for the amendment can be found in for example paragraphs [0039] and [0057].

These amendments are not believed to have introduced new matter into the application.

I. Claims 1, 2, 5-16, 18-30, 57, and 58 have been rejected under 35 U.S.C. § 112, second paragraph

Claims 1, 2, 5-16, 18-30, 57, and 58 have been rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the applicant regards as the invention. In particular, the rejection states "In claims 1 and 2, initially it is claimed that an N-glycan comprising GlcNAcMan5GlcNAc2 is produced. Then later in the claim, an oligosaccharide substrate is used to produce a desired N-glycan, with the desired N-glycan having the oligosaccharide branch Man α1,3 (Man α1,6) Manβ1,4-GlcNAβ1,4-GlcNAc-Asn. It is unclear whether the oligosaccharide branch Man α1,3 (Man α1,6) Manβ1,4-GlcNAβ1,4-GlcNAc-Asn is added to GlcNAcMan5GlcNAc2, or whether it is added to a different substrate. If Man α1,3 (Man α1,6) Manβ1,4-GlcNAβ1,4-GlcNAc-Asn is not added to GlcNAcMan5GlcNAc2, then it is unclear what purpose GlcNAcMan5GlcNAc2 has in the method."

Man α1,3 (Man α1,6) Manβ1,4-GlcNAβ1,4-GlcNAc-Asn is the description of the oligosaccharide core structure of the product produced by taking GlcNAcMan5GlcNAc2 (the oligosaccharide substrate) and digesting it with a mannosidase enzyme that is capable of hydrolyzing *in vivo* an oligosaccharide substrate comprising either or both a Manα1,3 and Manα1,6 glycosidic linkage. Mannosidase II is such an enzyme. It can convert the oligosaccharide substrate GlcNAcMan5GlcNAc2 to the product GlcNAcMan3GlcNAc2. GlcNAcMan3GlcNAc2 comprises the Man α1,3 (Man α1,6) Manβ1,4-GlcNAβ1,4-GlcNAc-Asn core structure. Figure 1B shows the conversion of GlcNAc2Man5GlcNAc2 to GlcNAcMan3GlcNAc2. *See* also paragraph [0032] and Figure 36A.

Mannosidase IIx has an enzymatic activity similar to the enzymatic activity of mannosidase II but has a different substrate specificity. Mannosidase IIx can convert the oligosaccharide substrate Man6GlcNAc2 to the product Man4GlcNAc2. Man4GlcNAc2 comprises the Man α1,3 (Man α1,6) Manβ1,4-GlcNAβ1,4-GlcNAc-Asn core structure. *See* also paragraph [0035] and Figure 36B.

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Mannosidase III has an enzymatic activity similar to the enzymatic activities of mannosidases II and IIx but has a substrate specificity that differs from both mannosidase II and mannosidase IIx. Mannosidase III can convert the oligosaccharide substrate $Man_5GlcNAc_2$ to the product $Man_3GlcNAc_2$. $Man_3GlcNAc_2$ has the Man α1,3 (Man α1,6) Manβ1,4-GlcNAβ1,4-GlcNAc-Asn core structure. *See* also paragraph [0039] and Figure 36C.

The claims have been amended to help clarify what the method entails. In light of the above, reconsideration of the rejection is requested.

CONDITIONAL PETITION

Applicant hereby makes a Conditional Petition for any relief available to correct any defect in connection with this filing, or any defect remaining in this application after this filing. The Commissioner is authorized to charge deposit account 13-2755 for the petition fee and any other fee(s) required to effect this Conditional Petition.

Respectfully submitted,

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